

# Image Analysis and Enhancement: General Methods and Biomedical Applications

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## Abstract

General methods of image processing, analysis and enhancement and their biomedical applications developed by the scientific school of the Laboratory of Mathematical Methods of Image Processing of the Faculty of Computational Mathematics and Cybernetics of Lomonosov Moscow State University are reviewed. The suggested general methods and algorithms of image quality enhancement for image resampling and super-resolution, ringing artifact reduction, image sharpening, image denoising, and image registration are described. Image analysis methods based on Hermite projection method, Gauss-Laguerre functions and the use of phase information are presented. We describe and review the developed methods for medical imaging tasks solution, including problems in histology, color Doppler flow mapping, ultrasound liver fibrosis diagnostics, CT brain perfusion, Alzheimer's disease diagnostics, dermatology, chest X-ray image analysis, live cell image registration, tracking, segmentation and synthesis. The paper illustrates the basic research idea of the effectiveness of the hybrid approach when we jointly use classical mathematical methods and deep learning approaches.

**keywords:** image analysis, image processing, image enhancement, medical images, cell microscopy images, mathematical methods, convolutional neural networks, hybrid methods.

## 1 INTRODUCTION

The article deals with the most interesting general image processing, analysis and enhancement tasks and their applications in biomedicine, which were the subject of research at the Laboratory of Mathematical Methods of Image Processing of the Faculty of Computational Mathematics and Cybernetics of Lomonosov Moscow State University. The methods and algorithms developed by the participants of the school while solving these problems are presented. This year the laboratory celebrates its 15th anniversary, but its roots go back to the traditions of the scientific school of the outstanding mathematician and founder of the Faculty of Computational Mathematics and Cybernetics Andrey N. Tikhonov.

The structure of the paper is as follows. Section 2 describes the works in image quality estimation and enhancement. The tasks of image resampling and different artifacts suppression are considered. The problems concerned with the Hermite functions-based technique and its application to the different image analysis tasks are presented in Section 3. Section 4 presents problems and algorithms in different biomedical applications. A short Conclusion ends the article.

## 2 IMAGE ENHANCEMENT AND IMAGE QUALITY ESTIMATION

The objective of image enhancement is to process an image so that the result is more suitable than original image for a specific application. This is especially important for biomedical applications when it is not possible to acquire images of necessary quality.

## 2.1 Image Resampling and Super-Resolution

A series of algorithms have been developed for image resolution enhancement. It includes both single-frame and multi-frame algorithms.

### 2.1.1 Single-frame super-resolution

For the problem of image resampling, also known as single-frame super-resolution, the research was carried out in two directions. In the first direction, a classical mathematical model for image resampling was considered. The image resampling problem was posed as an inverse problem to the image downscaling model. Tikhonov regularization method with a Total Variation stabilizer [56] was used to solve this ill-posed problem:

$$z_\alpha = \arg \min_z (\|Az - u\|_2^2 + \alpha TV[z]),$$

where  $u$  is the given low-resolution image,  $A$  is the downscaling operator,  $TV[z]$  is the stabilizer in the form of the total variation functional, and  $\alpha$  is the regularization parameter.

The total variation functional works as the prior knowledge about the image.

The result heavily depends on the choice of the regularization parameter. In [77], a relation between total variation values of corresponding low- and high-resolution images was set, and an explicit choice of the regularization parameter was suggested.

The second direction was based on machine learning approach, particularly on convolutional neural networks. Instead of developing new convolutional neural network models, we focused on the improvement of existing algorithms. Proper data preparation is as important as a good network model. In [68], a way to construct the training dataset for the problem of image resampling with noisy input has been proposed. In [46], an application of convolutional neural networks for retinal image upscaling was investigated. In [69], it was shown that applying Zero Component Analysis helped to achieve better results at edges and textured areas.

We have also developed several edge-directional image resampling algorithms with adaptive weights that are chosen according to local gradient features, and interpolation kernels that are learned using pairs of low- and high-resolution images of the target class [76, 79].

### 2.1.2 Multi-frame super-resolution

Multi-frame super-resolution is a reconstruction of a single high-resolution image from several low-resolution observations. Unlike single-frame super-resolution, the amount of available data is much more informative, and there is no need to use the prior information for high-resolution image reconstruction. Thus, the classical approach based on the image downscaling model is still widely used. It is posed as a set of equations: find a high-resolution image that gives the low-resolution observations after applying motion and downsampling operators, and it is often formulated as a minimization problem similar to single-image resampling:

$$z_\alpha = \arg \min_z \left( \sum_{k=1}^K \|AF_k z - u_k\|_2^2 + \alpha TV[z] \right),$$

where  $u_k$  are  $K$  input low-resolution images,  $F_k$  are corresponding motion operators.

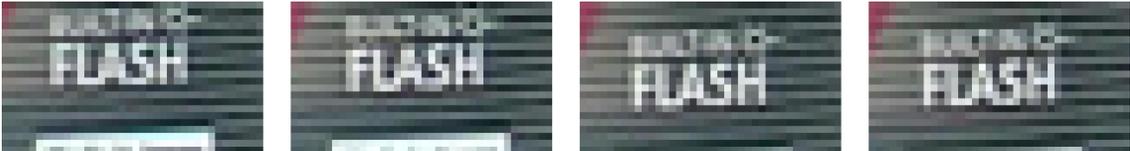
The most challenging problems become functional minimization and finding motion operators  $F_k$  which is a mapping between low-resolution images with sub-pixel accuracy (this problem is called optical flow estimation). Our research was focused on developing algorithms for computationally efficient non-iterative reconstruction from a series of images and video sequences.

In [49], we have addressed a problem of finding optical flow when illumination changes between frames. We have proposed a modification of Kanade-Lucas algorithm based on using partial derivatives of image intensity. In [50], a non-iterative algorithm was proposed for

solving the inverse problem. The idea was to adaptively find an inverse convolution kernel using self-similarity property and use it to find the high-resolution image directly.

In [73], weighted median filter was proposed for non-iterative reconstruction of a high-resolution image. Pixels from low-resolution images were mapped to a high-resolution grid, then an interpolation was performed using weighted median with Gaussian weights. This approach significantly improved the results for noisy images and images with erroneously estimated motion.

Fig. 1 demonstrates the results for the proposed super-resolution algorithms.



a) Low resolution images



b) Box interpolation



c) Single-image resampling using Total Variation regularization [56]



d) Multi-frame super-resolution using fast deconvolution and weighted median filter [73]



e) High quality multi-frame super-resolution

**Fig. 1** An example of super-resolution results for a real image sequence by the proposed algorithms. 16 low-resolution images were used to reconstruct a high-resolution image with 2x upsampling ratio

## 2.2 Ringing Artifact

Detection and suppression of the image Gibbs oscillations (ringing artifact) was one of the main topics of our research during many years. The ringing artifact is caused by high-frequency information corruption or loss. It appears as waves or oscillations near strong edges. The most known examples are its appearance in MRI images, in various image enhancement methods (deblurring, sharpening, upscaling, etc.), in JPEG2000 compression. We started with designing classical mathematics methods for ringing suppression (TV regularization) and ringing level estimation by edge profile analysis [42, 70, 71, 107] and their use for adaptive image deblurring and combined linear resampling method with ringing control [43, 45] and for the deringing of MRI medical images [130].

Later different methods for ringing detection and image deringing based on sparse representation approach were developed [122, 123, 124]. The ringing detection and suppression algorithms were based on construction of the synthetic dictionary that is used to represent ringing effect as a sum of blurred edge and pure ringing component. We compared the methods of joint dictionaries learning, and separate learning of natural images dictionary and pure ringing dictionary. It was found that the first approach was more appropriate for low level of ringing while strong ringing is better suppressed by the separate learning method.

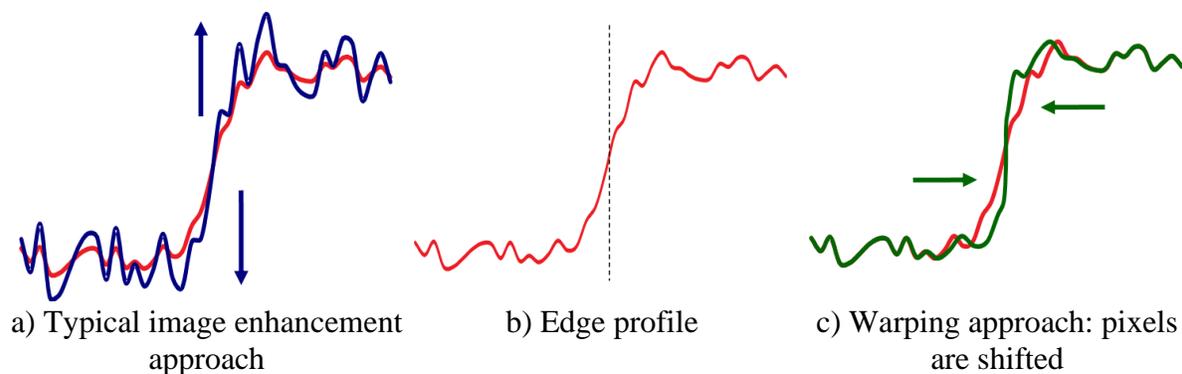
Last years we worked on hybrid method of deringing [96, 97] based on joint use of classical methods and deep learning.

## 2.3 Image Sharpening by Grid Warping

Image deblurring has been one of the challenging image processing problems for a long time. The problem is that the blur kernel and information about the noise type and level are not

known and have to be estimated. Non-uniformity of image blur, errors in blur kernel and noise estimation negatively affect the quality of the image deblurring algorithms.

We have proposed an approach based on grid warping algorithm to sharpen the edges by making the edge transient areas thinner without corrupting them (see Fig. 2) [84]. Compared to the classical image deblurring approach, the grid warping approach does not change the noise level, does not introduce ringing artifact and does not need the blur kernel. Instead, it needs only the approximate level of image blur.

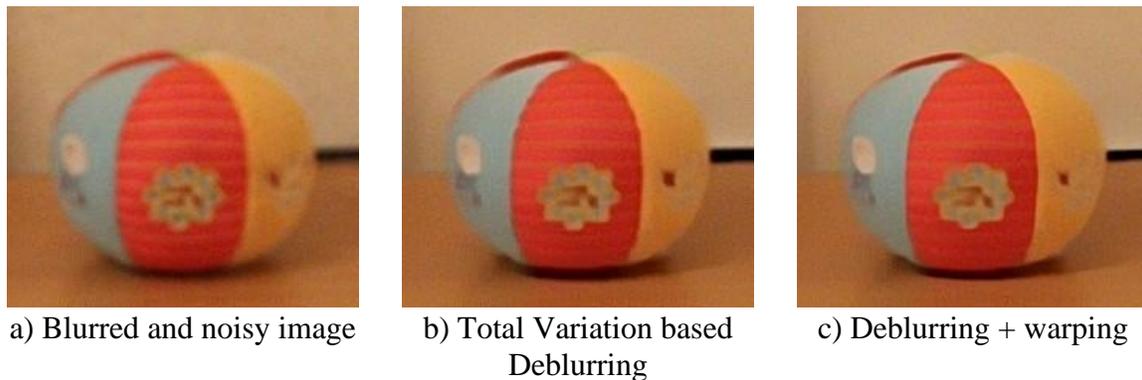


**Fig. 2** The idea of edge sharpening by grid warping

Since sharpening by grid warping affects only edges while keeping textured areas intact, the best scenario is to use the proposed warping algorithm as a post-processing step after existing image enhancement algorithms [47, 51, 82]. An example is shown in Fig. 3.

In [81], an accurate and robust edge width estimation method based on Gaussian edge model and unsharp mask analysis is proposed for the problem of parameter choice for the grid warping algorithm. Computationally efficient implementation of the proposed algorithm is proposed in [19]. In [78], a method to choose the parameters of the grid warping algorithm by a convolutional neural network is suggested. In [44, 48], a volumetric implementation of the

proposed algorithm is presented. An improvement of the grid warping algorithm in corner areas is presented in [75].



**Fig. 3** An example of applying the proposed warping algorithm as a post-processing step after image deblurring

The software implementation of the proposed algorithm is available at the authors' website <https://imaging.cs.msu.ru/en/soft>.

## 2.4 Image Denoising

It is still right that image denoising is the state of art challenge for researchers. Image noise reduction is an important problem in different areas of applications where we need to remove noise components preserving at the same time useful information. Image denoising has common approaches based on the assumption that the noise is additive white Gaussian noise (AWGN). Nevertheless, in many applications there are special types of noise and even combinations of different noise types like in CT, ultrasound, etc.

Two methods for noise reduction in CT images were introduced in our Lab in [117]: 3D extension of fast rank algorithms (Rank-2.5D) and 3D extension of a non-local means algorithm (NLM-2.5D). Image denoising algorithms using local Hermite projection method were also developed [61, 62]. An improvement of BM3D image denoising and deblurring algorithm by Generalized Total Variation was proposed in [74]. Method of low noise image

construction from a sequence of multiple noisy images using weighted nuclear norm minimization was suggested in [83].

Many of the existing state-of-the-art image denoising methods for AWGN are based on convolutional neural networks (CNN). In many cases they give better results than classical prior-based mathematical methods. Nevertheless, one cannot fully rely only on CNN based methods. Their results strongly depend on the used training dataset and even small differences in the input data can give an unpredictable output disturbance. To deal with the possible practical instability of CNN-based methods new hybrid denoising methods that included some combinations of CNN-based and classical denoising methods were suggested. These combinations of methods can give better results, but nevertheless additional problems arise. Along with the high uncertainty level of setting CNN hyperparameters there is also an uncertainty in the choice of parameters of classical methods. Thus, a no-reference automatic estimation of the parameters of classical filtering methods is needed. To solve this problem, we suggested a multiscale method for automatic choice of the denoising parameters. Parameter optimization is done in the ridge areas, when we can analyze their appearance on the difference between original noisy and filtered image (so-called method noise image). If this difference is irregular, then the filtering strength can be increased. If regular components appear on method noise, then the filtering strength is too large. We use mutual information closely connected with conditional entropy for the analysis and consider images corrupted with Gaussian-like noise with small correlation radius. Rfidge detection approach based on Hessian matrix eigenvalues analysis is used for estimation of sizes and directions of image characteristic details (see Section 2.4.1).

The AWGN denoising algorithms with this automatic choice of parameters were developed for NLM and LJNLM-LR methods [63], nonlinear diffusion [65], total variation [60], BM3D [64], Perona-Malik [80], and Weighted Nuclear Norm [125] methods.

Hybrid AWGN denoising methods using automatic choice of the classical algorithms were presented in [37, 52].

Hybrid method for Poisson noise reduction was presented in [21].

#### 2.4.1 Multiscale image ridge detection

Ridge detection is the key moment in the mentioned above image denoising methods.

We use the following algorithm of ridge detection based on the idea that Laplacian filter can indicate the presence of ridge. Let us define:

$$L^\sigma(x, y) = \sigma^2 \cdot I(x, y) * G_\sigma(x, y),$$

$$G_\sigma(x, y) = \frac{1}{2\pi\sigma^2} e^{-\frac{x^2+y^2}{2\sigma^2}},$$

where  $I(x,y)$  is intensity of the source image. The multiplier  $\sigma^2$  in the equation for  $L^\sigma(x, y)$  is used to equalize Laplacian filter response at different scales. So, if we increase both the image and  $\sigma$  by same factor the filter response will be same for the corresponding points. We also note that the differentiation of  $L^\sigma(x,y)$  is equivalent to convolution of the source image  $I(x,y)$  with corresponding Gaussian function derivative. Let  $L_{xx}^\sigma(x, y)$ ,  $L_{xy}^\sigma(x, y)$ ,  $L_{yy}^\sigma(x, y)$  be second derivatives of  $L^\sigma(x,y)$  by the corresponding variables. Modulus of Laplacian  $\Delta L^\sigma(x, y)$  has maximum in the central point of ridge of  $2\sigma$  width. To find ridge direction we build a Hessian matrix:

$$H^\sigma(x, y) = \begin{pmatrix} L_{xx}^\sigma(x, y) & L_{xy}^\sigma(x, y) \\ L_{xy}^\sigma(x, y) & L_{yy}^\sigma(x, y) \end{pmatrix}.$$

Then eigenvector  $\vec{v}^{\sigma}(x, y)$  corresponding to the lowest eigenvalue of  $H^{\sigma}(x, y)$  will be directed along the ridge.

For multiscale ridge detection we use different values of  $\sigma \in \{\sigma_1, \dots, \sigma_n\}$ ,  $\sigma_i = \sigma_0 \cdot \nu^{i-1}$ .

Calculating  $\Delta L^{\sigma_i}(x, y)$  and  $\vec{v}^{\sigma_i}(x, y)$  corresponding to  $\sigma_i$ , we find the characteristic ridge size  $s(x, y)$  and direction  $\vec{v}(x, y)$  as:

$$s(x, y) = \arg \max_{\sigma_i} (|\Delta L^{\sigma_i}(x, y)|),$$

$$\vec{v}(x, y) = \vec{v}^{s(x, y)}(x, y).$$

An approach for color ridge detection was suggested in [22]. It was also found that the use of anisotropic diffusion in the multiscale ridge detection method substantially improved the ridge detection quality [59].

The hybrid methods based on convolutional neural networks and multiscale ridge detection were proposed for biometric image segmentation [102, 121] and human image matting [104].

## 2.5 Image Registration

Image registration tasks are very actual for general image analysis problems and they arise in many biomedical applications. Our specific image registration algorithms in histology (see Section 4.1) and cell image analysis (see Section 4.8.2) are described later.

At the same time we worked on general rigid registration methods acceleration. In [119] an iterative exclusion of heavily mismatched contour points, followed by rectification of the parameters for the rest of the points was suggested. Here a special structure of the histological images that contain elliptical gland slices enabled us to find corresponding ellipses on the fixed and moving images.

A series of works for the estimation of the optimal image downsampling coefficient to speed up the mutual entropy maximization method for rigid registration was done. The estimation algorithm used the analysis of the dependence of parameters of the fast bidirectional empirical mode decomposition method on scaling [17, 18, 36, 106].

## 2.6 Image Quality Assessment

Image quality assessment algorithms usually produce a single value that represents image quality. But this value does not explain why one image is better than another one. Common factors that affect image quality are insufficient image reconstruction or introducing artifacts like ringing effect, blur or noise amplification. An analysis of image artifacts may help improve image quality assessment.

We have proposed a way to find image areas where typical artifacts of image enhancement algorithms are likely to appear. We have introduced the concept of *basic edges* — sharp and strong edges that are distant from other edges [72].

Two areas near basic edges are identified: BEP (basic edge profile) — the area including the basic edge itself with a small neighborhood up to  $1/2$  of edge width, and BEN (basic edge neighborhood) — the area with a distance between  $1/2$  and  $2$  of edge width from the basic edge. BEP area is used to analyze blur effect while BEN area is used to detect ringing effect.

The proposed algorithm is illustrated by Fig. 4. It consists of the following steps:

1. Canny edge detection with zero thresholds and scale parameter  $\sigma$  corresponding to the expected artifact size.
2. Edge masking: edges with gradient magnitude less than  $1/2$  of magnitude of the surrounding edges are considered as masked. These edges usually belong to image textures and are not suitable for artifact analysis.

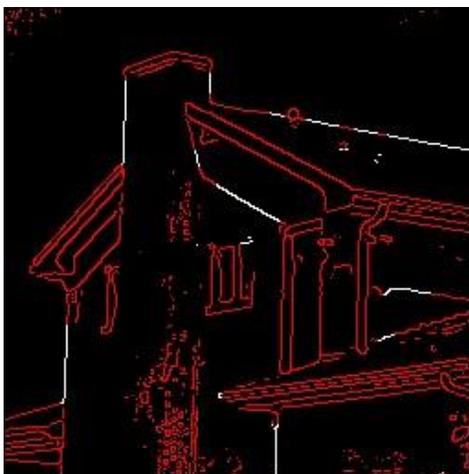
3. Finding basic edges: non-masked edges that are distant from other non-masked edges.
4. Finding BEP and BEN areas.



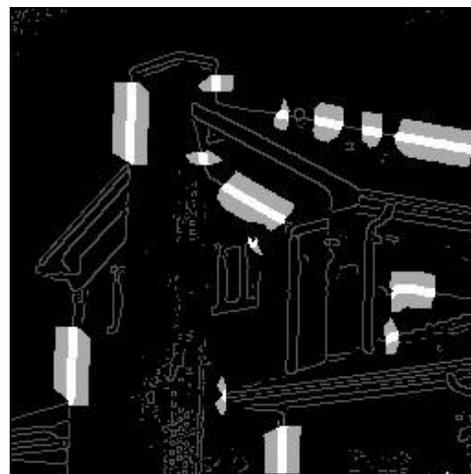
a) Input image



b) Edge detection + edge mask map  
Masked edges are denoted by blue color



c) Basic edges (white color) and non-basic edges (red color)



d) Basic edge areas: BEP (white areas) and BEN (gray areas)

**Fig. 4** An illustration of the basic edge areas detection algorithm

The software implementation of the proposed algorithm is available at the authors' website <https://imaging.cs.msu.ru/en/soft>.

In [109], we have proposed a short reference image quality metric based on basic edges and Gauss-Laguerre projection method (see Section 3.2 for details). We have defined the modular angular edge coherence metric that is computed in the BEP and BEN regions of the image.

The proposed metric was evaluated in the image sharpening task.

In [13] we have also introduced a metric based on the modified structural similarity index to enhance non-local means image denoising algorithm. The potential of this metric with physically justified weighting function for each component was demonstrated.

### 3 IMAGE ANALYSIS

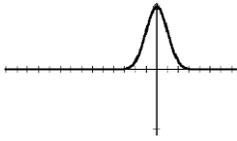
#### 3.1 Hermite Projection Method

The choice of Hermite functions for signal and image analysis is not accidental. When studying and solving operator equations of mathematical physics (let us formally write such an equation as  $Az = u$ ) and especially, when solving inverse problems for these equations, a lot of information corresponding to the physics of the phenomena under study is contained in the eigenfunctions of the operator  $A$  for the considered setting of the function space (or the operator  $A^*A$  in the case of a non-self-adjoint operator). This determines the use of Hermite functions being the eigenfunctions of the Fourier transform from  $L_2(\mathbb{R})$  to  $L_2(\mathbb{R})$  in signal processing and analysis methods, because in many cases these methods are based on the use of the Fourier transform. Moreover, the very definition of the Fourier transform from  $L_2(\mathbb{R})$  to  $L_2(\mathbb{R})$  in the classic books by Norbert Wiener and Edward Titchmarsh is given using Hermite functions.

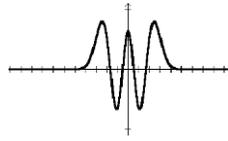
Technically, the orthonormal in  $L_2(\mathbb{R})$  Hermite functions  $\psi_k(x)$  can be calculated as

$$\begin{aligned}\psi_0(x) &= \frac{1}{\sqrt[4]{\pi}} e^{-x^2/2}, \\ \psi_1(x) &= \frac{\sqrt{2}x}{\sqrt[4]{\pi}} e^{-x^2/2}, \\ \psi_n(x) &= x \sqrt{\frac{2}{n}} \psi_{n-1}(x) - \sqrt{\frac{n-1}{n}} \psi_{n-2}(x).\end{aligned}$$

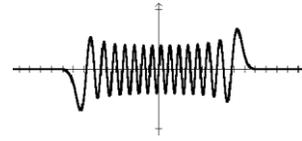
They can be also defined using the derivatives of the Gaussian function. The examples of graphs of Hermite orthonormal functions are given in Fig. 5-7.



**Fig. 5**  $\psi_0$  function



**Fig. 6**  $\psi_4$  function



**Fig. 7**  $\psi_{29}$  function

Note that the similarity of the behavior of internal oscillations of Hermite functions to the behavior of trigonometric functions is not accidental. Asymptotically, the values of the Hermite functions tend to the functions  $\cos(x)$  and  $\sin(x)$  depending on the parity of the number.

For the case of “symmetrical” Fourier transform defined as

$$\mathfrak{F}f = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{+\infty} f(x)e^{-i\lambda x} dx,$$

the following equation holds

$$\mathfrak{F}\psi_n = (-i)^n \psi_n,$$

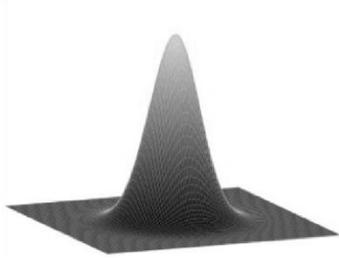
so both functions  $\psi_n$  and their Fourier transforms  $\mathfrak{F}\psi_n$  are equally computationally localized in spatial and frequency domains.

Generally speaking, the expansion into the series of Hermite functions (Hermite projection method) gives an alternative for the concept of “frequency”. And these functions are “time-frequency” computationally localized instead of non-localized in space trigonometric functions, so the Hermite projection method is a good candidate for the substitution of the discrete Fourier transform in signal processing and analysis tasks of different dimensions.

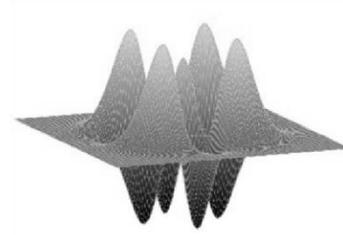
Two-dimensional orthonormal in  $L_2(\mathbb{R}^2)$  Hermite functions  $\psi_{n,m}$  are defined in terms of one-dimensional functions as

$$\psi_{n,m}(x, y) = \psi_n(x)\psi_m(y).$$

The examples of graphs of two-dimensional orthonormal Hermite functions  $\psi_{n,m}$  are shown in Fig. 8 and Fig. 9.



**Fig. 8**  $\psi_{0,0}$  function



**Fig. 9**  $\psi_{2,2}$  function

By the Hermite projection method, we mean the expansion of a function into a Fourier series of Hermite functions

$$f(x) = \sum_{k=0}^N c_k \psi_k(x),$$

where  $\psi_k(x)$  are one-dimensional Hermite functions orthonormal on  $L_2(\mathbb{R})$ ,  $c_k$  – Hermite coefficients:

$$c_k = \int_{-A}^A f(x)\psi_k(x)dx.$$

For the fixed  $N$ , the limit  $A$  is defined so that all functions  $\psi_k, k \leq N$  are computationally orthogonal to each other. So the expansion is computationally orthogonal.

The idea of the Hermite projection method was introduced in [41]. The method was used for the diffraction data analysis [6, 53] and as a substitution of the Fourier discrete transform for image filtering [39]. Then it was used for the tasks of texture parametrization [40], image deblocking [67] and image database retrieval [35]. Fast Hermite projection method was presented in [38].

In collaboration with Professor Srdjan Stanković and Professor Irena Orović from University of Montenegro the method was applied in a series of works in the area of time-frequency analysis [115, 116].

Hermite functions and Hermite projection method were also used in biometrics for iris image analysis, iris image key points detection [90] and comparison [87, 89].

In [91, 92] the approximation of Fourier transform of  $f(x) = \sum_{k=0}^{\infty} c_k \psi_k(x)$  using Hermite functions (HFT) was defined:

$$HF_{f,n}(x) = \sum_{k=0}^n c_k (-i)^k \psi_k(x),$$

and the synthesis of HFT phase and magnitude of different images using Hermite projection method was suggested. The theorem of uniqueness of signal reconstruction to within a scale factor from phase only information of its HFT was given. The algorithm of signal reconstruction from HFT phase only information was proposed [89] (Fig. 10).

In [88], the Hermite projection phase only correlation (HPPOC) function was introduced for image matching. If two images are similar, their HPPOC function gives a distinct sharp peak. If two images are not similar, the peak value drops significantly. The advantage of HPPOC function is especially noticeable in small size image matching, and HPPOC in iris recognition for iris key points matching was proposed.

### 3.2 Gauss-Laguerre Functions for Image Analysis

Let us consider a family of complex orthonormal and polar separable functions defined in polar coordinates  $r, \gamma$ :

$$\Psi_n^\alpha(r, \gamma; \sigma) = \psi_n^{|\alpha|}(r^2/\sigma) e^{i\alpha\gamma}.$$



**Fig. 10** The iterative image reconstruction using HFT phase-based reconstruction algorithm; (b) is the 2D projection image approximation of the image (a); (c)-(h) are the results after different number of iterations

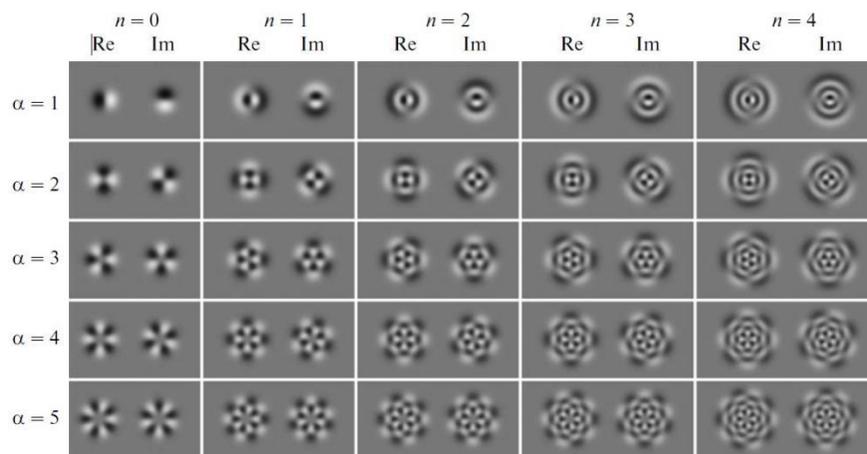
These functions are called Gauss-Laguerre circular harmonic functions (CHFs), are referenced by integers  $n = 0, 1, \dots$  (referred by radial order) and  $\alpha = 0, \pm 1, \pm 2 \dots$  (referred by angular order), and their radial profiles are Laguerre functions:

$$\psi_n^{|\alpha|}(x) = \frac{1}{\sqrt{n! \Gamma(n + \alpha + 1)}} x^{\alpha/2} e^{-x/2} L_n^\alpha(x), \quad L_n^\alpha(x) = (-1)^n x^{-\alpha} e^x \frac{d}{dx^n} (x^{n+\alpha} e^{-x}).$$

The real and imaginary parts of  $\Psi_n^\alpha(r, \gamma)$  ( $n = 0, 1, \dots, 4; \alpha = 1, 2, \dots, 5$ ) are illustrated in Fig. 11.

Using the Gauss-Laguerre CHFs we developed the projection method for local image descriptors construction and keypoints detection. The projection method was based on the local image expansion into the set of Gauss-Laguerre CHFs in the support region of a keypoint [110]. Using the properties of these function and their connection to 2D Hermite functions we significantly sped up the computation of the descriptors. Moreover, we proposed a special numerical method to reduce the computational complexity of the proposed projection methods [111]. The proposed approach demonstrated its effectiveness compared to SIFT

descriptors in the task of estimation of homography matrix for several images taken from different viewpoints. The method was further generalized to be used with color images [54] where we proposed to use a special form of color image gradient for differential operations on color images instead of converting them to grayscale. Such approach demonstrated that the use of color information for keypoints descriptors construction for both color and grayscale keypoints detection usually enhance the image matching quality.



**Fig. 11** Real and imaginary parts of  $\Psi_n^\alpha$  ( $n = 0, 1, \dots, 4; \alpha = 1, 2, \dots, 5$ )

### 3.3 Phase-Based Methods

The phase often contains more important information about the signal than the magnitude. Generally, the global phase and the local phase are used. The global phase usually means the Fourier transform phase of the whole signal while the local phase is the phase of the local transform (windowed Fourier transform, wavelet transform, etc.).

We propose two phase-based methods for key points matching based on the use of phase information in local areas around the key points, and we use these methods for iris key points matching. In the first method the Hermite projection phase only correlation (HPPOC) function is calculated [88, 89]. If the key points are from the same pattern of iris texture, the HPPOC function has a sharp peak, while in the case of different eyes, the HPPOC function does not

have a sharp peak. The second method uses the phase congruency information in the points around iris key points for image matching [99, 100].

## 4 BIOMEDICAL APPLICATIONS

### 4.1 Histology

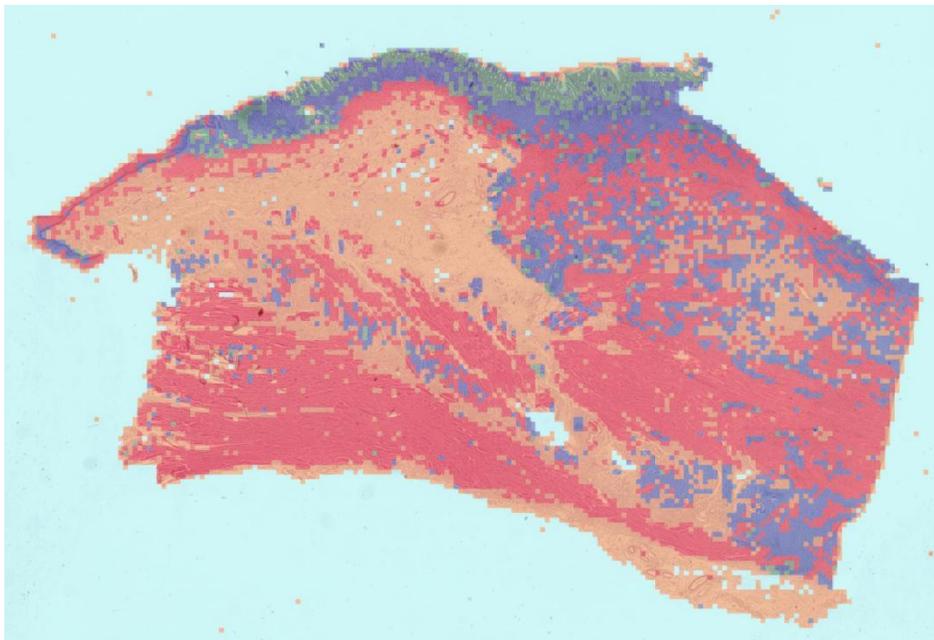
One of the fastest growing areas of medicine with a wide range of applications for image processing and computer vision is the field of histology, which recently due to the widespread use of new generation scanners transformed into digital pathology. Modern scanners can get images of the entire glass with the tissue sample, the resolution of such whole slide images (WSIs) can be  $100k \times 100k$  pixels and even more, the size of these images is calculated in gigabytes.

The complexity and variety of histological structures open a big number of tasks of automatic image analysis including segmentation, registration, content-based retrieval and many more. Due to the variability of histological structures, it is almost impossible to analyze these images with “classical” mathematical methods, thus we focused on development of deep learning methods and hybrid methods.

Apart from using existing public datasets we created our own dataset PATH-DT-MSU [27, 86], where within the S1 subset we collected and annotated histological images with normal and open glands and proposed a new CNN architecture for gland segmentation. The more difficult task that is in demand for histologists is to perform object gland segmentation. We developed an original hybrid method of trainable active contours [28], the key feature of which was deep integration of classical active contour and the CNN models.

In our research we also focused on the problem of reduction of time needed to annotate the image and developed a method for fast annotating of biomedical images [29]. This hybrid method unites the KNN model with CNN and allows to speed up the gland segmentation process by up to 10 times.

The obvious problem for processing WSIs with neural networks is their incredible resolution which negatively impacts the performance. The process of annotating WSIs is also too time-consuming. On the other hand, the big advantage of working with WSIs is the ability to look at the whole image and analyze the layers of tissue. Thus, the easiest way to segment WSIs and identify the layers is the approach of segmentation by classification (Fig. 12), which we used in [30].



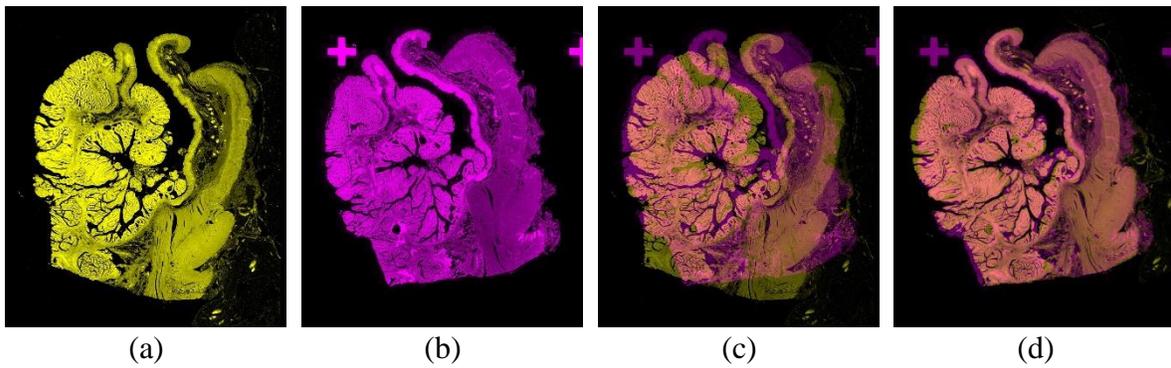
**Fig. 12** Visualization of whole slide histological image segmentation through classification with semitransparent color mask. Different colors correspond to different tissue types

Another important task for the WSIs is image registration. In medical practice it is often necessary to jointly analyze differently stained histological sections. However, during the preparation of slides the tissues undergo deformations and image registration is highly required. The importance of the task is confirmed by the international challenge on Automatic

Non-rigid Histological Image Registration (ANHIR) that was organized in frame of International Symposium on Biomedical Imaging in 2019 [7], where we took part in the preparation of the challenge datasets and development of the evaluation metrics and worked with our colleagues from the Czech Republic and Spain. The challenge became continuous and online and by the time of writing this paper had 42 submitted methods. The dataset collected and marked up during the preparation became a standard benchmark for evaluating the histological image registration approaches. Using the ANHIR dataset we developed our own affine histological image registration approach [101] (see Fig. 13) that is a zero-shot CNN-based method that outperforms the competing affine registration methods by the time of writing this paper.

The main problems of working with WSIs are the problem of transmitting the collections of images between histologists and the presence of a large number of different incompatible file formats. To solve these problems, we developed PathScribe (<https://pathscribe.ru>) – a new cloud-based multi-platform software tool for working with WSIs. Since autumn 2022 PathScribe is being used for educational purposes at the Faculty of Medicine, Lomonosov Moscow State University. PathScribe is being actively developed both as educational and scientific tool.

The work was performed in collaboration with Professor Pavel G. Malkov and Ilya A. Mikhailov from the Medical Research Center, Lomonosov Moscow State University.



**Fig. 13** Affine registration results. Source (a) and target (b) images are depicted in yellow and purple color scale respectively for better visualization clarity. The overlay of unregistered images is shown in (c), registration result overlay is shown in (d).

## 4.2 Color Doppler Flow Mapping

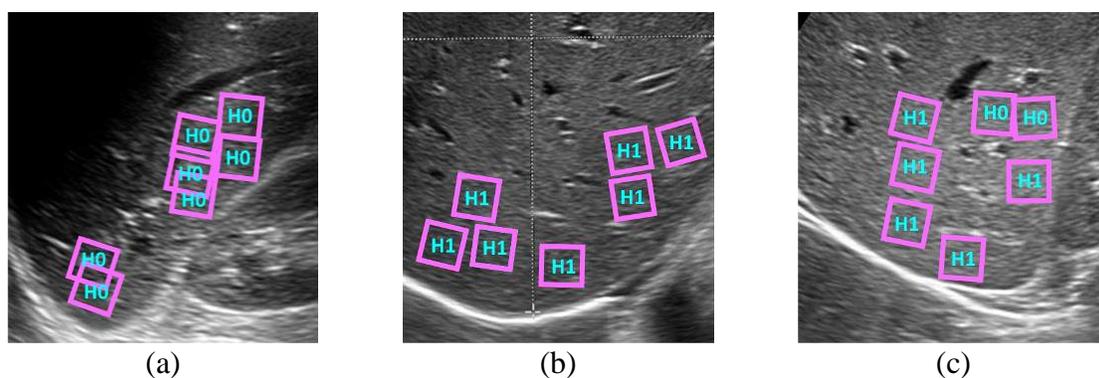
Computational problems of ultrasound heart diagnostics were investigated in collaboration with Petrovsky National Research Center of Surgery of Russian Academy of Medical Sciences. They included building a three-dimensional dynamic model of left cardiac ventricle [128] and a series of computational methods for Color Doppler Flow Mapping image data unwrapping. It includes graph-cut based methods [127] and regularization methods [131]. Later the cross-frame connection was suggested for optimization of the algorithms [126]. An original complex phase preliminary filtration was also used to suppress a false-aliasing artifact and to improve the results [129]. Flow variances were used as the weight coefficients in the minimization of energy function. For the comparison a test data series were also constructed. They used specially designed anatomic 3D left ventricle region model for the simulation of the blood flow. Experiments showed that the suggested preliminary filtration and cross-frame weights significantly improve the quality of unwrapping. The work was performed in collaboration with Academician Valeriy A. Sandrikov and the head of laboratory Tatyana Yu. Kulagina.

### 4.3 Ultrasound Liver Fibrosis Diagnostics

Another interesting task in biomedical image analysis is the problem of analyzing ultrasound images of liver to perform liver fibrosis diagnostics. The main feature of the task is the fuzziness of the image structure itself and the diffuseness of the fibrosis disease. These restrictions do not allow to use the entire image for analysis, which reduces the problem to applying texture analysis of small regions of interest in the image with clear texture (Fig. 14). As a result, we proposed a semi-automatic method for fibrosis diagnostics based on texture analysis and machine learning [55].

The main disadvantage of ultrasound images due to the physics of the process is the presence of specific multiplicative noise in the image, called speckle noise. Therefore, we conducted a separate study to assess the influence of the speckle noise level and the use of denoising methods on texture analysis [25]. The final result of the study was a complex algorithm for diagnosing liver fibrosis using ultrasound images, elastography data and the proposed new methods for speckle denoising, and texture analysis with machine learning [26].

The work was performed in collaboration with Professor Julius R. Kamalov from Petrovsky National Research Center of Surgery.



**Fig. 14** Visualization of regions of interest selection for texture analysis and corresponding class prediction for the problem of liver fibrosis diagnostics. H0 stands for the normal control prediction, H1 stands for fibrosis. Ground truth values are H0 for (a), (b) and H1 for (c).

## 4.4 CT Brain Perfusion

The quantitative analysis of the blood flow in the brain tissue is one of the important problems in neurosurgery. It arises when diagnosing acute ischemic stroke. This problem can be solved using computed tomography perfusion imaging. There are various methods for extracting quantitative characteristics of cerebral blood flow from CT perfusion data, which differ in degrees of their noise resistance. More noise-resistant methods enable the reduction in radiation doses when conducting the examination of the patient. Hence, the development of noise-resistant methods is an important problem. The classical approaches to solve this problem are based on singular value decomposition (SVD) method with Tikhonov regularization and methods of total variation (TV) minimization. These methods were outperformed by our new algorithm that is using total generalized variation minimization [57], but later we found that the better method evaluating the quantitative characteristics of cerebral blood flow is based on the regularization using the projection onto a set of monotonic functions while minimizing the functional of total generalized variation (TGV) [58]. The work was performed in collaboration with Professor Vasily A. Lukshin and Academician Dmitry Yu. Usachev from Burdenko National Medical Research Center of Neurosurgery.

## 4.5 Alzheimer's Disease Diagnostics

In the last decade, computer-aided early diagnostics of Alzheimer's Disease (AD) and its prodromal form, Mild Cognitive Impairment (MCI), has been the subject of extensive research. Attempts to recognize the early stage of the disease and incorporate this recognition into general population screening have led to the task of determination the stage of Alzheimer's disease by analyzing 3D images of human brain obtained within MRI, DTI, PET modalities. In our research we proposed several CNN-based approaches taking into account the specifics of the problem. The solutions were based on applying transfer learning [1], constructing 3D

convolutional neural networks [23] and optimization of architectures and the number of classification model parameters [24]. The work was performed in collaboration with the Laboratory of Professor Jenny Benois-Pineau from University of Bordeaux, France.

## 4.6 Dermatology

Different image analysis and processing tasks in dermatology were investigated. They include the problems of border extraction of epidermises, derma and subcutaneous fat in high-frequency ultrasonography [105], dermatological image hair removal using grid warping approach [85], ridge-based method for pemphigus diagnosis on immunofluorescence images [16], epidermis area detection in immunofluorescence microscopy [14], and adaptive dermatological image denoising using Hermite projection method for non-local means algorithm [15].

The work was performed in collaboration with Professor Natalia V. Makhneva from M.F. Vladimirsky Moscow Regional Research and Clinical Institute.

## 4.7 Chest X-Ray Image Analysis

A deep learning-based approach for determination of the radiation level and a system of automatic out-of-distribution detection for pulmonary X-ray image analysis have been proposed in [11, 12, 94]. Both algorithms evaluate the radiation level according to the number of distinctly visible thoracic vertebrae. A CNN-based method for chest X-ray tuberculosis diagnostics has been developed. It was used to investigate the influence of dataset thinning according to the predicted image radiation level on its performance. The study has revealed an increase of diagnostics accuracy after discarding of images of extreme radiation levels from the training dataset. The works were performed in collaboration with Professor Liubov E. Parolina from the National Medical Research Center for Phthisiopulmonology and Infectious

Diseases, Moscow, and the scientists from the E.N. Andreev Scientific Practical Phthiology Center of Sakha Republic, Yakutsk, Russia, headed by Egor S. Prokopev.

## 4.8 Cell Image Analysis

One of the most quickly developing areas of computer science is microscopy cell image analysis. In the last several years we have developed several methods and algorithms that help biologists to analyze their data.

### 4.8.1 Live cell image analysis

In recent years, we developed several image analysis methods that were applied to practical problems of live cell images analysis. Namely, we developed a method for tracking of intercellular foci in fluorescence microscopy image sequences that was used for investigation of the mechanisms underlying the movement of nuclear bodies using experimentally induced interphase prenucleolar bodies (iPNBs) [5]. The cornerstone of iPNB tracking approach in this work was the use of cell image registration technique [112] (see Section 4.8.2 for details) that allowed compensating the global motion of the nucleus and analyze only the local iPNBs motion patterns. We further developed the proposed approach and published it as a research protocol in a book chapter [108] accompanied with the source code to be applicable in a wider range of biological practical tasks. The work was performed in collaboration with the group of Professor Eugene V. Sheval from the A.N. Belozersky Institute Of Physico-Chemical Biology, Lomonosov Moscow State University.

### 4.8.2 Cell image registration

In clinical practice it is frequently necessary to analyze several images of the same organ simultaneously. The images can be obtained in different modalities, at different time points, or for different subjects. The problem is actual for different types of data such as MRI,

sonography, radiography, computer tomography, microscopy and others. The problem of simultaneous image analysis is that the images of different modalities, time points or patients are prone to deformations as they are not acquired simultaneously, or undergo deformations while image acquisition process. Thus, image registration is the essential problem in medical and biological image analysis.

The registration problem is also actual for cryo-electron microscopy (cryo-EM) images. The registration of the single particles in cryo-EM images is one of the crucial steps of the 3D model reconstruction pipeline. In 2017, we developed the correlation-based approach for the registration of the single particle cryo-EM projection images [2] that enabled one to register the images more than 300 times faster than the greedy iterative approach. The complexity of the task is related to the extreme level of noise in the cryo-EM images.

In microscopy live cell image analysis, the registration problem arises in the analysis of intercellular foci motion in living cells. It is a complex problem as living cells are moving and deforming during the imaging process. The observed motion of subcellular foci consists of two components: local motion of the foci and global motion of the nucleus, which includes nucleus displacement and deformation. To determine information about the pure subcellular foci motion, the global motion of the nucleus needs to be compensated. This is usually done by means of image registration. In 2018, the non-rigid method for cell image registration in fluorescence microscopy image sequences was developed [112]. The core idea of the method is to use the well-founded physical model of non-linear elastic motion based on Navier equation which was applied for modelling the nucleus non-rigid motion. This work was started while Dmitry V. Sorokin was at the post-doc stay at the Centre of Biomedical Image Analysis, Masaryk University, Brno, Czech Republic in collaboration with Dr. Pavel Matula. The

obtained results allowed to overcome the global motion compensation problem and were used in the live cell analysis pipelines in the biological studies [5, 108].

We also developed a method for the non-rigid registration and non-uniform illumination compensation of the miniscope images of the rodent brain [103].

The registration problem is also the important part of histological image analysis [7, 101] (see Section 4.1).

### **4.8.3 Cell tracking and segmentation**

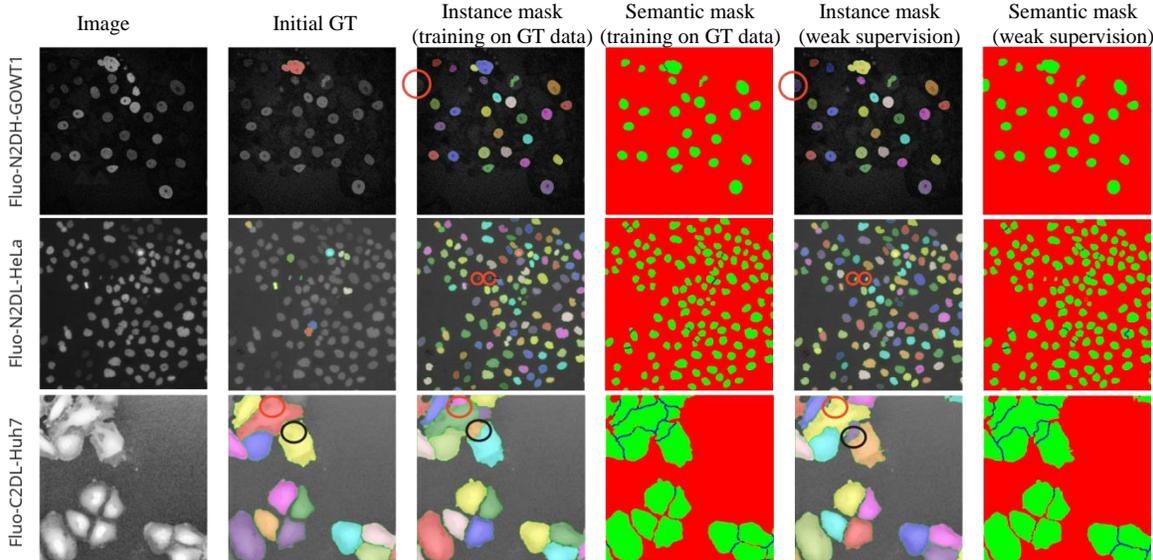
The segmentation of intercellular structures and small particles is another common task in live cell microscopy image analysis. We developed an approach for laser-induced structure detection in live cell nuclei images [33]. The approach is based on active contours and the results of the work were used for the development of the standard cell image registration evaluation dataset that was further used in [112] and following works on this topic for the evaluation of the cell image registration methods.

The automated tracking of cells and subcellular structures in fluorescence microscopy images are extremely important tasks. The former enables the analysis of the life cycle of individual cells through migration, growth and proliferation. The latter allows analyzing, for example, the motility of fluorescentstained actin filaments [34] for which we developed the automatic tracking method. This analysis of filaments motion is important for muscle functions research.

The cell tracking recently formed the individual well-known area of the biomedical image analysis field. The Cell Tracking Challenge (CTC) first organized in 2012 significantly stimulated the research in this field and caused the development of many widely used segmentation and tracking methods. For example, the wide-spread U-Net segmentation CNN architecture was initially developed for this challenge.

In 2018, in collaboration with the group of Professor Yasushi Okada (University of Tokyo) we developed the cell tracking approach for 2D epifluorescence microscopy image sequences [31] that allowed to overcome the tracking result of the existing semi-automatic method previously used for this data. The approach was extended in [32] by introducing the weakly-supervised segmentation stage which further improved the results.

In 2022, we developed the weakly-supervised segmentation approach [4] that extended the original sparse segmentation annotation using the tracking annotation and cell image registration approach. The proposed approach took the third place in Fluo-C2DL-Huh7 dataset of the Cell Segmentation Benchmark of CTC in April 2022. The results of the proposed weak supervision approach compared to the conventional learning using the provided GT only are presented in Fig. 15.



**Fig. 15** Segmentation results for Fluo-N2DH-GOWT1, Fluo-N2DL-HeLa, and Fluo-C2DL-Huh7 datasets of CTC. The red circles indicate the improvement of using the proposed in [4] weak supervision approach: the frame boundary cells are segmented in comparison with the baseline segmentation method trained on GT data. The white circles show the improvement for touching cells. The black circles show the mistakes made in both approaches for Fluo-C2DL-Huh7 dataset.

#### 4.8.4 Cell image synthesis

Objective benchmarking of biomedical image analysis methods requires a lot of diverse image datasets accompanied by reference annotations. For many problems the creation of such datasets is really time consuming especially because of creation of reference annotations that has to be done with qualified experts manually. Another way to obtain the required data is to synthesize realistic images of the same class. If the synthesized data is realistic, it enables one to generate as much data as possible and the reference annotation in this case is generated inherently by the image synthesis method. Another valuable outcome of such data can be its usage for training the machine learning approached in the cases where there is a lack of real biomedical data with reference annotations. Thus, development of image synthesis methods becomes a very important task.

In cooperation with research groups from Czech Republic, Spain and France we developed the image synthesis methods for generating the realistic images of cells with filopodial protrusions with the inherently generated ground truth segmentation masks and tracking annotation. The methods are presented in a series of works. The initial proof-of-concept approach was presented in [113] where we modeled the filopodial growth and elastic motion of the individual cell which was further converted to the 3D image sequences using the virtual microscopy techniques. In [114], the work was improved by incorporating more complex models into the method resulting in the online application called FiloGen that allows one to generate the image sequences of different types of filopodial cells controlling the filopodia development, motion and virtual microscopy parameters. The further work [98] was concentrated on multiple filopodial cells interaction. The work on FiloGen was started while Dmitry V. Sorokin was at the post-doc stay at the Centre of Biomedical Image Analysis, Masaryk University, Brno, Czech Republic in collaboration with Dr. Martin Maška.

The data obtained using FiloGen was used for developing the state-of-the-art filopodial cells segmentation and tracking methods for 3D fluorescence microscopy images in [66] and [8].

Another example of realistic biomedical image synthesis method is the cryo-EM and cryo-ET image synthesis developed in [3].

#### 4.8.5 Blinking fluorescence

Blinking fluorescence super-resolution microscopy enables to surpass the diffraction limit without the use of complex equipment. A variety of algorithms based on different approaches have been developed in the past years for the problem of sharp high-resolution image reconstruction: SOFI, MUSICAL, SPARCOM, COLORME, DLBI, 3B etc. Given an input image sequence  $\{\bar{y}_t\}_{t=1}^N$ , the broadest statement of the problem is to find at least one of the following: its high-resolution preimage  $\{\bar{x}_t\}_{t=1}^N$ , the blinking average or some kind of the blinking map (e.g., pixel variance or higher moments). The blur and downscaling operator  $K$  and the noise variance  $\sigma$  may also be available. In most cases an input image is considered to be  $\bar{y}_t = K\bar{x}_t + \bar{b} + \bar{n}_\sigma$ , where  $\bar{b}$  is the constant background and  $\bar{n}_\sigma$  is the Gaussian noise.

In [95], a regularization probability-based approach for high resolution image reconstruction has been presented and analysis of its performance in various conditions has been carried out.

The problem is defined as follows:

$$\{\bar{x}_t^*\}_{t=1}^N, \bar{\mu}^*, \Lambda^*, \bar{\sigma}^* = \arg \min_{\substack{\{\bar{x}_t\}_{t=1}^N \geq 0, \\ \bar{\mu} \geq 0, \Lambda > 0, \bar{\sigma} \geq 0}} \sum_{t=1}^N \left[ \begin{aligned} & (\bar{y}_t - K\bar{x}_t)^T (\text{diag}(\bar{\sigma}))^{-1} (\bar{y}_t - K\bar{x}_t) + \\ & + (\bar{x}_t - \bar{\mu})^T \Lambda (\bar{x}_t - \bar{\mu}) - \\ & - \ln |\Lambda| + \ln |\text{diag}(\bar{\sigma})| \end{aligned} \right] + \\ + \alpha \|\bar{\mu}\|^2 - \beta \ln |\Lambda| + \text{Tr}(\Gamma\Lambda),$$

where  $\bar{\mu}$ ,  $\Lambda$ ,  $\bar{\sigma}$  are the blinking average, the matrix inverse of the blinking covariance matrix and the Gaussian image noise pixel-wise variance respectively,  $\alpha$ ,  $\beta$  are the regularization parameters and  $\Gamma$  is the regularization matrix.

Being a representation of the image blinking map and due to the lack of the non-blinking background, the most desired is the main diagonal of the blinking covariance matrix. To find it a family of computationally efficient algorithms has been proposed recently and the importance of the regularization term becomes prominent for them. The influence of various regularization terms  $\Omega(x)$ , including the RED (regularization-by-denoising) approach based on NLM (non-local means) and TV (total variation) denoising methods, upon the solutions of the problem defined as

$$\min_{r_{\bar{x}} \geq 0} \frac{1}{2} \|\mathbf{R}_{\bar{y}} - \mathbf{K} \text{diag}(r_{\bar{x}}) \mathbf{K}^T - \sigma \mathbf{I}\|_F^2 + \lambda \Omega(r_{\bar{x}}),$$

where  $\mathbf{R}_{\bar{y}}, r_{\bar{x}}, \sigma$  are the input image sequence covariance matrix, its preimage pixel-wise variance and the Gaussian noise variance respectively, has been considered in [93].

The work on blinking fluorescence super-resolution microscopy has been performed in collaboration with the Laboratory of Professor Yasushi Okada from the University of Tokyo.

## 5 CONCLUSION

Fast growing of artificial intelligence methods in image analysis and applications enables to solve general and applied problems in many cases much more efficiently than classical mathematical methods. It also includes tremendous progress in biomedical image analysis. Nevertheless, many problems arise. The methods become much more dependent on the used dataset, the algorithms may become less robust, etc. Our practice shows that the optimal solutions usually can be found by the hybrid methods when we jointly use artificial intelligence methods and classical mathematical methods of image processing, analysis and enhancement.

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